Short communication

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Rabbit myostatine gene polymorhism (c.747+34C>T and c.194A>G) as a marker for meat production

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Abstract

Myostatin gene (MSTN or GDF-8) affects both, the amount and composition of muscle fibers. MSTN plays a key role in muscle growth and it has applications in breeding and animal husbandry. Polymorphic variants of this gene are associated with growth rates and carcass yield of different species. This study is focused on two single nucleotide polymorphisms: MSTN SNP c.747+34C>T and MSTN SNP c.194A>G and their effect on selected production traits. Genomic DNA was extracted from whole blood. Polymorphic variants of MSTN gene were detected using PCR-RFLP method. Association studies revealed significant positive effect of allele T (c.747+34C>T) and allele G (c.194A>G) on meat performances in tested rabbit population.

Keywords: association studies, GDF-8, meat production, MSTN, rabbit

Introduction

Research focused on the association studies of candidate genes is a step for the knowledge of the genetic basis of economically important traits (Óviló et al., 2016). A candidate gene approach is based on the variability within genes coding proteins involved in metabolic pathways related to production traits such as growth, body weight, or carcass traits. Rabbit meat is highly rated for its nutritional and dietetic properties as it contains less fat and cholesterol and more protein compared to other meat types. Thus, in the last decade studies of rabbit candidate genes connected to meat quality have become a field of great interest. Myostatin gene (MSTN or GDF-8) plays a key role in muscle growth as it controls the proliferation of muscle precursor

cells and affects the amount and composition of muscle fibers (McPherron, 1997). Thus, it has applications in breeding and animal husbandry (Thomas et al., 2000). Polymorphic variants of this gene are associated with growth rates and carcass yield of cattle (Sellic et al., 2007; Gill et al., 2009), sheep (Hickford et al., 2009), goat (Zhang et al., 2012) and chicken (Zhang et al., 2011; Dementeva et al., 2017). Rabbit myostatin gene consists of three exons and two introns. Six single nucleotide polymorphisms (SNP) were identified so far (Fontanesi et al., 2011; Sternstein et al., 2014). The present study was carried out to investigate the association between MSTN gene (SNP c.747+34C>T and c.*194A>G) and meat production parameters in rabbits.

Materials and methods

For the presented study, 70 animals (males, synthetic meat line P-91) from Research Institude for Animal Production in Nitra were used in total. Animals were fed *ad libitum* by commercial pelleted feed and had free access to water. Animals were maintained under controlled microclimate conditions (humidity 60 ±5%, temperature 17 ±3 °C, photoperiod 14 hours light / 10 hours darkness, maximum air flow rate 0.05 ms^{-2}). Animal experiments were authorized by MPSR No. SK28004 and Ro 2058 / 06-221 / c. To determine the relationship between myostatin gene polymorphic variants and meat production, whole blood samples for DNA isolation were collected and birth weight of animals, live weight on day 7, 14, 21, 28, and 84 and average daily gain were measured and calculated. At the end of the experiment animals were slaughtered and carcass yield was measured. Genomic DNA was extracted by standard salting out extraction protocol (Miller, 1988). Primers were designed according to Fontanesi et al. (2011). Two SNPs (c.747+34C>T and c.*194A>G) in the rabbit MSTN gene were screened using PCR restriction fragment length polymorphism (PCR-RFLP) according to Fontanesi et al. (2011). Statistical analysis was performed using statistical software SPSS for Widows, Release 6 a Statistix for Windows, Version 8. For average daily gains and live weights of rabbits, Pearson correlations were calculated, moreover live weight growth for genotypes was measured in days by linear and non-linear regression with respect to age.

Results and discussion

In realized experiment 70 rabbits for SNP c.747+34C>T and 60 rabbits for SNP c.*194A>G were genotyped. After *PCR-RFLP* using two restriction endonucleases *AluI* (c.747+34C>T) and *TaaI* (c.*194A>G) both alleles of SNPs were detected. For SNP c.747+34C>T frequency of allele C was 0.52 and frequency of allele T was 0.48. Fontanesi et al. (2008) reported the frequencies of the C and T alleles of SNP at c.747+34C>T to be 0.51 and 0.49 in their rabbit population (commercial paternal line). The same alleles displayed frequencies of 0.67 and 0.33, respectively, in M91 and P91 rabbit lines (Rafayová et al., 2009), in Giant Grey and New Zealand White rabbit (Sternstein et al., 2014) were allelic frequencies 0.33 (C) and 0.67 (T). In contrast, Markowska et al. (2010) reported frequencies of T and C allele at 0.38 and 0.62 in Polish and White Flemish Giants Rabbits, Bindu et al. (2012) 0.43 and 0.57 in a pooled population of New Zealand White and Soviet Chinchilla and their crosses

(Fontanessi et al., 2008) at c.747+34C>T detected the frequency of allele C (0.51) and frequency of allele T (0.49), similar to observed experimental population where the frequency of allele C was slightly over the frequency of the T allele. Abdel-Kafy et al. (2016) reported allele frequencies in APRI rabbits line 0.27 (C) and 0.73 (T). Rafayová (2010) confirmed significant differences in favor of animals with TT genotype in myostatin polymorphism c.747+34C>T with T allele frequency (0.67) and allele C (0.33). For SNP c.*194A>G observed allele frequencies were 0.67 (A) and 0.33 (G), what is similar to findings of Adbel-Kafy et al. (2016). An adequate level of heterozygosity with respect to the marker was also confirmed by the calculated value of the heterozygosity coefficient at the levels of 0.49 resp. 0.44. Based on the effective amount of the allele, it can be concluded that the alleles have been balanced in the population. A good level of heterozygosity occurred at the mean value of the polymorphic information content (0.37, resp. 0.34).

From the obtained results it is clear that animals with genotype TT (SNP c.747+34C>T) showed the highest average values in all monitored indicators (Table 1).

Table 1. Associations between MSTN c.747+34C>T and production traits in rabbit

n = 70	CC (n = 21)	CT (n = 31)	TT (n = 18)	Genotype		
Parameters	Mean ±SD	Mean ± SD	Mean ± SD	CC - CT	CC - TT	CT - TT
BW0 [g]	62.3 ±9.8	65.2 ±10.3	74.1 ±11.3	2.9	11.8**	8.9*
BW7 [g]	185.4 ±24.8	187.6 ±29.8	196.8 ±25.2	2.2	11.4**	9.2
BW14 [g]	298.5 ±39.4	320.8 ±41.3	338.2 ±39.4	22.3*	39.7**	17.4
BW 21 [g]	438.2 ±43.2	443.5 ±42.4	468.3 ±41.3	5.3	30.1**	24.8**
BW 28 [g]	523.7 ±162.7	562.7 ±73.6	595.6 ±64.4	39*	71.9**	32.9
BW 84 [g]	2392.5 ±215.9	2384.3 ±197.3	2546.7 ±225.7	8.2	154.2**	162.4**
ADG [g]	28.5 ±2.8	28.4 ±2.4	30.3 ±3.1	0.1	1.8	1.9*
CY [%]	54.3 ±4.1	56.3 ±3.8	58.9 ±4.1	2	4.6*	2.6

BW – body weight; SD – standard deviation; ADG – average daily gain; CY – carcass yield; * P<0.05; ** P<0.01; ***P<0.001.

Also at SNP 194 A>G, allele G shown to be considerably beneficial for the tested rabbits in all monitored parameters (Table 2). Qiao et al. (2014) confirmed that myostatin plays a significant role in rabbit growth and development and thus can be considered as a candidate gene for meat production. Sternstein et al. (2014) found a link between the MSTN SNP polymorphism c.373+234G>A and a fresh meat composition. But they did not notice any significant effect of polymorphisms of MSTN. In their experiments they focused on qualitative indicators not quantitative.

Abdel-kafy et al. (2016) studied the effect of three mononucleotide polymorphisms of myostatin (c.713T>A, c.747+34C>T and c. * 194A>G) on rabbit growth markers. For polymorphisms c.747+34C>T and c. 194A>G, they found allele frequencies identical to the frequencies in presented work. In SNP c.713T>A, there was no significant correlation between genotype and growth markers.

Table 2. Associations between MSTN 194 A> G and production traits in rabbit

n = 60	AA (n = 28)	AG (n = 24)	GG (n = 8)	Genotype		
Parameters	Mean ±SD	Mean ±SD	Mean ±SD	AA-AG	AA-GG	AG-GG
BW0 [g]	63.8 ±9.3	66.4 ±11.6	76.2 ±12.8	2.6	12.4**	9.8**
BW7 [g]	176.6 ±21.7	191.5 ±28.5	198.7 ±26.3	14.9**	22.1**	7.2
BW14 [g]	299.6 ±41.2	328.4 ±42.4	337.4 ±38.7	28.8**	37.8**	9
BW 21 [g]	442.8 ±44	452.3 ±43.6	472.4 ±42.4	9.3	29.6**	20.1
BW 28 [g]	526.4 ±158.4	574.6 ±74.2	591.4 ±53.3	48.2**	48.2**	16.8
BW 84 [g]	2401.2 ±220	2398.2 ±198.4	2562.8 ±231.5	3	161.6**	164.6**
ADG [g]	27.8 ±2.8	27.7 ±3.9	29.6 ±4.5	0.1	1.8	1.9**
CY [%]	53.6 ±4.1	57.2 ±3.7	58.6 ±5.6	3.6**	5**	1.4

BW – body weight; SD – standard deviation; ADG – average daily gain; CY – carcass yield; * P<0.05; ** P<0.01; *** P<0.001.

Conclusions

Single nucleotide polymorphisms in myostatin gene of rabbit (c.747+34C>T and c.*194A>G) are significantly associated with body weight of rabbits and carcass yield (P<0.05). Animals with genotype TT and GG show higher values in all measured parameters compared to CC and CT, resp. AA and AG genotypes. Presented results, in accordance with the results of other authors, confirm the role of myostatine gene in animal production. Analyzed single nucleotide polymorphisms could be used for selection of animals with higher meat performances.

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